PART C - QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)

1.0   INTRODUCTION AND OVERVIEW

The purpose of this chapter is to outline the quality assurance (QA) and quality control (QC) efforts required to support development of human exposure and dissipation data that are required under 40 CFR 158.390 and described in Part B of these Guidelines.

This Chapter is divided into five sections: (1) Introduction; (2) Pre-field Laboratory Considerations; (3) Field Study Considerations; (4) Laboratory Analysis of Collected Samples; and (5) Good Laboratory Practices (GLP).

The <u>Pre-field Laboratory Considerations</u> section covers those experiments associated with developing an analytical method for the analyte(s) of interest and validating its expected accuracy, precision, and specificity in a specific sample matrix. Requirements in this area include determining the laboratory recovery efficiency, limits of detection and quantification, and working concentration range of the method. Optional, but recommended, requirements include determining the stability of the samples under the expected conditions of storage as well as the recovery from fortified samples exposed under simulated field conditions.

The <u>Field Study Considerations</u> section covers the major aspects of proper sample collection and validation as specified in the study design. Sample collection requirements include, but are not limited to, calibrating application and sampling equipment, observing and documenting important aspects of study conduct, and ensuring that samples are properly handled and stored. Sample validation requirements include the selection of an appropriate control site(s) and the preparation and collection of field recovery samples.

The <u>Laboratory Analysis of Collected Samples</u> section covers the need for regular laboratory instrument maintenance and calibration programs, the use of standard calibration curves to correct for day-to-day instrument variability, and the preparation and analysis of concurrent laboratory recovery samples to verify that the losses during extraction, cleanup, analysis, etc. are within acceptable limits as defined by the pre-field method validation work.

The <u>Good Laboratory Practices (GLP)</u> section covers the procedures required by field and laboratory personnel to document the access, location, condition, and handling of each sample from the time of collection through analysis.

The <u>Data Reporting</u> section describes the treatment of nonquantifiable values, the presentation of field and laboratory recovery data, and the appropriate data calculation and correction procedures.

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2.0   PRE-FIELD LABORATORY CONSIDERATIONS

Analytical methods and sample collection procedures should be developed and validated before the field monitoring portion of a study is undertaken. Many aspects of the overall study design, such as the nature of the active ingredient and the end-use product in question, the proposed sampling technique(s) and duration, the anticipated time and conditions of sample storage, etc. can influence the method development and validation process. Study design issues are discussed in Part B, Chapter 2 of these guidelines and should be considered for the study in question before and during method development and validation. In addition, the relevant toxicological endpoints (acute and chronic) for the compound in question should be considered during the method development and validation phase of the study so that acceptable margins of exposure can be demonstrated in the event that exposure samples yield values below the limit of detection (LOD)/limit of quantification (LOQ). Sufficiently low LOQ and LOD are expected so that the Agency can avoid having to estimate the margin of exposure (MOE). (See Part D, Chapters 1 and 2.)

Although it may be desirable to include a complete report of the method development and validation work in the protocol, it is not practical in most cases because this work may continue for several months subsequent to the completion of the protocol. However, as indicated in Part B, Chapter 2 - Study Design, the protocol should include a brief description of the anticipated analytical method to be employed and the proposed LOQ and LOD for each collection medium. The protocol should also include information on the stability of the analyte in or on the collection medium, if available. The final report of the study submitted to EPA must include a complete report of the method validation work.

2.1   Analytical Method Development

Analytical methods should be developed before field samples are collected. The method must be specific for the analyte of interest at an appropriate LOQ level. The necessary LOQ for any assessment will depend upon the toxicological endpoint of interest. At a very minimum, the LOQ must be sufficient to assess exposures below the no observable effect level (NOEL) based on the toxicological endpoint or its equivalent mg/kg for the appropriate dosing frequency, which may be daily. It is usually desirable and recommended to be able to quantify exposures well below the NOEL using a study design factor (i.e., historically referred to as a safety factor). One approach to estimate a LOQ for dermal exposures is to be able to quantify the NOEL delivered as a dermal dose deposited uniformly onto the 1.92 m² skin area (approximately 20,000 cm²) of a standard 70 kg/person. The average dermal deposition density at this condition calculated by Equation C-1 reduces to approximately 3.5 NOEL ug/cm² for the standard person.

$$\text{Avg. Deposition Density, } \mu g/cm^2 = \frac{[\text{NOEL, mg/kg}] \times [\text{body mass, kg}] \times [10^3 \ \mu g/mg]}{[\text{body surface area cm}^2]} \qquad \text{(Eq. C-1)}$$

A good target LOQ must be below the average deposition density presented in Equation C-1 because of: (1) the uncertainty in estimating a NOEL for the dermal route of exposure (e.g. the dermal NOEL may need to be estimated from oral NOEL times a coefficient representing the difference between dermal and gastrointestinal (GI) absorption); (2) differences between typical frequency and duration of use patterns and those actually assessed; (3) the reduction in collection time if assessments cannot be run for the full exposure period per day; (4) likely variations in chemical deposition densities over skin locations; (5) the potential impact of conservative interpretations of results below the LOQ (i.e., non-zero assumptions of nonquantifiable or nondetectable samples); and (6) uncertainties associated with toxicological endpoints such as inter- and intra-species variations. These study design factors (often described as a safety factor) do not add safety to the NOEL but encourage setting a target LOQ for dermal (or other field) exposure assessments that will be below the minimal concentration noted above by factors of 10, 100, or more.

$$\text{Dermal LOQ, } \mu g/cm^2 = \frac{[\text{dermal deposition density, } \mu g/cm^2]}{[\text{study design factor}]} \qquad \text{(Eq. C-2)}$$

Air sampling may or may not be targeted at the same NOEL, depending upon various factors such as differences in absorption rates or in metabolism between exposure via the air versus dermal routes. Estimates of a maximal LOQ for air samples can be calculated in basically two steps: first by calculating the airborne concentration corresponding to the most appropriate NOEL (Equation C-3), then by calculating the LOQ of the air sample's liquid extract (Equation C-4). As with the other maximal LOQ examples discussed herein, it is desirable to target an air sample LOQ below the maximal airborne LOQ by a study design factor of 10, 100, or more.

$$\text{Air Concentration, } \mu g/L = \frac{[\text{NOEL, mg/kg] x [body mass, kg] x } [10^3 \, \mu g/mg]}{[\text{air volume inhaled during activity, L}]} \qquad \text{(Eq. C-3)}$$

Should a NOEL air concentration be available directly from some toxicity test, then only Equation C-4 need be used.

$$\text{Airborne LOQ, } \mu g/L = \frac{[\text{air concentration, } \mu g/L]}{[\text{study design factor}]} \qquad \text{(Eq. C-4)}$$

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Two additional steps may be needed. Air volume sampled is determined by flow rate and time of the activity or the exposure being assessed. Keep in mind, the full duration of daily exposure may be different from the duration of time the air sample is actually collected, although as a first approximation they can be assumed to be equal.

$$\text{Air volume, L} = [\text{flow rate, Lpm}] \times [\text{sample collection time, minutes}] \qquad \text{(Eq. C-5)}$$

Should the toxicity data base yield a NOEL concentration in terms of ppm in the vapor state, a molar conversion must be calculated as shown in Equation C-6 based on the molecular weight of the analyte.

$$\text{Air Concentration, } \mu\text{g/L} = \frac{[\text{air concentration (ppm)} \times \text{molecular weight}]}{[24.45 \text{ L per mole at standard conditions}]} \qquad \text{(Eq. C-6)}$$

A similar approach can be applied to dislodgeable foliar residues (DFRs) by incorporating a transfer coefficient. A DFR corresponding to the NOEL can be estimated by first adjusting the dermal NOEL to some appropriate unit of activity, typically per hour, and then applying the corresponding transfer coefficient (e.g. Popendorf and Leffingwell, 1982). (See the discussion of transfer coefficients and alternative units of activity in Section D.2.5.)

$$\text{DFR at NOEL, } \mu\text{g/cm}^2 = \frac{[\text{NOEL, mg/kg}] \times [\text{body mass, kg}] \times [10^3 \ \mu\text{g/mg}]}{[\text{work day, hr}] \times [\text{transfer coefficient, cm}^2/\text{hr}]} \qquad \text{(Eq. C-7)}$$

Finally, the LOQ to quantitate this DFR sample will depend upon the foliar sample collected and the design factor applied, as shown below:

$$\text{DFR LOQ, } \mu\text{g/cm}^2 = \frac{[\text{DFR at NOEL}]}{[\text{study design factor}]} \qquad \text{(Eq. C-8)}$$

Again, it may be desirable to target an LOQ for field studies to be below the above maximal DFR LOQ value by a study design factor of 10, 100, or more.

The procedures to estimate LOQs for biological monitoring are similar in principle to those described above, but the details vary with the biological index being assessed.

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The analysis of urinary excretion is another laboratory problem similar to dosimetry except in an aqueous media. Quantitation requires an LOQ for the chemical/metabolite corresponding to the NOEL in the urinary volume over which the chemical/metabolite will be collected. In principle, there exists a consistent ratio between the mass of chemical exposure, the absorbed dose, and the amount of chemical or metabolite excreted. In reality, this ratio varies widely (ACGIH, 1990). Not only can the mass excreted per dose vary, but so can its rate of excretion and its concentration in the urine vary. Average urinary volume is 1.2 L/day (50 mL/hour), but this can easily vary ±3 fold and up to 4 or 5 fold under adverse circumstances of weather, fluid intake, metabolic and psychologic stress, medication, or disease.

For urinary monitoring to be a viable option, one must be able to anticipate either a proportionate amount of excretion over a given time frame following a single dose or a rate of excretion following a repeated daily dose. The first situation is applicable to rapidly metabolized chemicals; the latter, to more slowly metabolized chemicals. The general trend for pesticides is away from chemicals with long biological half lives, toward the more rapidly metabolized chemicals. Assuming that a ratio ($K_{excretion}$) can be defined between a predictable level (mg) of excreted chemical or metabolite at the NOEL (or ideally at any dose) as shown in Equation C-9, then the concentration that can be quantitated in the urine at this dose can be estimated from Equation C-10.

$$K_{excretion} = \frac{[\text{urinary excretion, mg}]}{[\text{NOEL, mg/kg}] \times [\text{body mass, kg}]} \qquad \text{(Eq. C-9)}$$

$$\text{NOEL, mg/kg} = \frac{[\text{urinary excretion, mg}]}{[K_{excretion}] \times [\text{body mass, kg}]} \qquad \text{(Eq. C-10)}$$

$$\text{Urine LOQ, } \mu g/mL = \frac{[K_{excretion}] \times [\text{NOEL, mg/kg}] \times [\text{body mass, kg}]}{[\text{study design factor}] \times [\text{urinary volume, L}]} \qquad \text{(Eq. C-11)}$$

This maximal LOQ can be greatly decreased if the chemical or metabolite in the urine is extracted and resuspended in another solvent, in which case this reduced extraction volume should be substituted for the urinary volume in Equation C-11. In addition to establishing a method with sufficient sensitivity, pre-field method development especially of a metabolite should also establish specificity from other natural or pharmacological sources. Collecting a pre-exposure sample from each individual (as discussed in Part B,

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Chapter 10) is a positive check on the adequacy of this aspect of pre-field method development, as well as the prior exposure status of participants.

The analysis of chemicals in blood can be treated in a manner identical to urine in Equations C-10 and C-11, if one can demonstrate or assume a similar ratio of blood content to dose exists for all dosing routes at equal points in time. It is desirable that the ratio between blood content and dose (k_{blood} in Equation C-12) be established for the most important environmental dosing route, although it is recognized that this toxicological data point is rarely available. The total blood volume used in Equation C-12 to derive k_{blood} would be the animal species tested; total blood volume in Equation C-13 would be for humans (which on average is 5 L but varies with weight, body build (fat-to-lean tissue ratio), and gender). Lacking any other measurable toxicologic parameter, any measured k_{blood} from a similar temporal dosing scheme could be used to establish a LOQ for blood at or below the NOEL using Equation C-13.

$$k_{blood} = \frac{[\text{blood concentration, } \mu g/L] \text{ x } [\text{total blood volume, L}]}{[\text{applied dose, mg/kg}] \text{ x } [\text{body mass, kg}] \text{ x } [10^3 \ \mu g/mg]} \qquad \text{(Eq. C-12)}$$

$$\text{Blood LOQ,} \ \mu g/mL = \frac{[k_{blood}] \text{ x } [\text{NOEL, mg/kg}] \text{ x } [\text{body mass, kg}]}{[\text{study design factor}] \text{ x } [\text{total blood volume, L}]} \qquad \text{(Eq. C-13)}$$

2.2   **Analytical Method Validation**

Analytical method validation establishes the performance of a particular method (e.g., the expected accuracy, precision, and specificity of a procedure for specific concentration ranges) within an analytical environment (e.g., within the laboratory that will perform the analysis of field samples). Method validation includes the analysis of a range of recovery samples for each matrix, including the LOQ. Performance criteria should include a demonstration of the capability to attain reproducible results when measuring analytes at the desired level of sensitivity for all substrates <u>prior</u> to the initiation of field studies. Seven samples per fortification level per matrix are required for method validation experiments. The completion of all validation work prior to the initiation of field studies is not mandatory, but it is recommended. Method validation experiments must be conducted under GLP standards.

<u>Minimally</u>, the analytical method validation must include the following:

- Establishment of the method's working concentration range of expected values from the field studies.

- Determination of detector response over a reasonable standard concentration range.

- Determination of the accuracy and precision of the method within the analytical environment through an experiment that should include the analysis of at least seven replicates of each fortification level indicated below for each substrate:

    -- The method limit of quantitation (LOQ),
    -- An intermediate concentration level (e.g., 10X LOQ),
    -- The maximum concentration of the validation range (e.g., 100-1000X LOQ), and
    -- Blank or control substrate.

In most instances, an accuracy value between 70 and 120 percent (average recovery) and a precision value less than or equal to 20 percent (coefficient of variation) demonstrates the analytical environment's capability to perform accurate and precise analysis. Note: The analytical method's accuracy and precision can also be handled on a case-by-case basis.

2.3 Other Validation Studies

Storage Stability. A storage stability study can be conducted prior to or in conjunction with a field study. Its purpose is to determine the stability of analyte(s) in or on appropriate sample matrices under storage conditions similar to those anticipated for storage of field samples. Conducting a storage stability study prior to study initiation may eliminate the need for generating storage recovery data during the field conduct of the study.

A storage stability study should include the following parameters:

- Preparation and analysis of at least three blanks, three low-level fortifications (2-10X the LOQ), and three high-level fortifications in the expected range of the field samples for each storage interval, including the longest interval planned for storage of field samples; and

- Storage of stability samples under the same conditions of storage as planned for the field samples (e.g., sample matrices or extracts, ambient temperature and/or frozen, etc.)

A storage stability study, preliminary or in conjunction with the field study, is optional if the field QA/QC samples are stored and analyzed with the actual field samples.

Pre-Trial Field Recovery Study. A pre-field (simulated) field recovery study is optional but is recommended. The purpose of a simulated field recovery study is to estimate the potential loss of analyte

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from sample collection devices, when subjected to environmental conditions (e.g., temperature, light, relative humidity, wind) and sample durations representative of those anticipated during collection of the actual field exposure samples. Because a simulated field recovery study is optional and is intended only to further minimize the risk to investigators, the Agency will not request that a specific number of samples be completed. However, it is recommended that a simulated field recovery study consist of preparation and analysis of at least three blanks, three low-level fortifications (2-10X the LOQ), and three high-level fortifications in the expected range of field samples. Laboratory incubators can be used to simulate anticipated field temperature and humidity conditions. If environmental conditions are anticipated to change during the exposure monitoring period, then a worst-case scenario should be simulated. Investigators should consider worst-case scenarios by providing data pertaining to the physical/chemical characteristics of the pesticide(s) being studied and the anticipated climate where the study is to be conducted when simulating worse-case scenarios.

2.4   Sample Collection Devices

During the pre-field phase of the study, investigators must give consideration to selecting the most appropriate sample collection device(s) and methodologies available for monitoring the pesticide(s) and use scenario(s) in question. Dosimeters and other sample collection devices must be durable enough to survive the physical stress and duration of the overall monitoring effort, including sample collection, transportation, and analysis. In addition, reasonable judgement should be used in the selection of sample collection devices to ensure that they do not reach their absorptive capacity (i.e., become saturated) during the sample collection process. Sampling media should be selected that do not contain substances that might interfere with the analysis. Pre-extraction may be required to remove coextractants and contaminating substances. Also, investigators must consider logistics pertaining to the preparation and storage of any dosimeter and/or monitor.

3.0   FIELD STUDY CONSIDERATIONS

Proper quality control and quality assurance measures during the field phase of a study are critical for ensuring the protection of volunteer subjects as well as the scientific validity of the study. Field QA/QC considerations can generally be divided into three areas: those pertaining to the protection of study participants, those pertaining to analytical field operations, and those pertaining to field data collection and documentation. Only the latter two subject areas will be discussed here.

The protection of study participants is achieved through compliance with the provisions of the Worker Protection Standard and informed consent. (See Part B, Chapter 2 - Study Design.) Analytical field QA/QC operations are oriented toward quantitatively tracking the residue of concern throughout the field

phase of a study (e.g., field recovery samples, optional travel recovery samples, fortifying procedures and the selection of a clean control site). Finally, critical elements for field data collection and documentation include: study site characteristics; application equipment and parameters; climatological data; sampling equipment and techniques; quality control and sample generation; dosimeter and sampler locations; human activity patterns; and sample storage and shipment.

3.1   Analytical Field QA/QC Operations

Analytical QA/QC considerations during the field trials of any study pertain to quantitatively tracking the residue of concern through the sample collection phase. The design of this aspect of the study is left to the discretion of the investigator as long as the stability of the compound is tracked from the field to the analytical phase. The most effective mechanism for completing this effort is through a properly executed field recovery regimen. Field recovery samples must be included in a study to allow the experimental data to be corrected for losses that occur during all phases of sample collection and analysis. Specifically, field recovery samples that are collected, handled, transported, and stored in conjunction with the experimental samples account for losses that occur during all phases of sample collection, sample handling and storage in the field, transportation from the field to the laboratory, and storage in the laboratory. Additionally, field recovery samples that are analyzed concurrently with the experimental samples account for residue losses that occur during sample extraction and analysis. Travel recovery samples can also be prepared in the field and shipped and stored with the experimental samples. The results of the travel recovery samples provide a basis for estimating the losses that occur during sample shipment and storage as opposed to those that occur during sample collection. The inclusion of travel recovery samples is optional.

3.1.1   Field Recovery of Human Exposure Samples

Field recovery refers to data generated to determine the loss of analyte from samples collected and fortified in the field, when subjected to the same environmental conditions (e.g., temperature, light, relative humidity, wind) and duration as field samples. Field recovery samples may also reflect the total losses that occur during sample collection, shipment, storage, and analysis.

Ideally, a separate set of field recovery samples should be collected for each work cycle (or activity period) monitored at each site. From a logistical standpoint, however, it is often more practical to collect one set of field recovery samples to represent all work cycles monitored at a given site on a given day. This approach is acceptable provided that the field recovery samples are collected in a manner that produces the most conservative estimates of recovery (e.g., collected during the highest temperature, wind, and/or relative humidity present during any of the work cycles monitored). It may also be acceptable to collect a single set of field recovery samples for all work cycles monitored at a given site over the course of a few days if the

environmental conditions are similar each day. This approach is only recommended for compounds that are very stable and in locations where the climate does not change appreciably from one day to the next during monitoring. The investigator who chooses this approach to generating field recovery data must demonstrate the stability of the compound as well as the day-to-day consistency of the climate at the study site(s).

A complete set of field recovery samples are used to represent all workers monitored for each sampling matrix during a work cycle irrespective of their particular job function. In addition, the number of field recovery samples collected during a work cycle is not influenced by the number of workers being monitored. A complete set of field recoveries should consist of three or more each of blank control samples, low-level fortifications, and high-level fortifications. The low- and high-level fortifications should be in the range of the anticipated level of the chemical on the substrate. If the highest expected level is more than 100X the lowest fortification level, it is recommended that a mid-level of fortification be included.

It is advisable to generate sufficient field recovery samples to be analyzed with the actual field samples to serve as concurrent laboratory recovery samples. At a minimum, a complete set of field recoveries would consist of the following, as applicable.

Inhalation (air) Sampling Matrices. An appropriate number of controls, low-level fortifications, and high-level fortifications should be prepared and analyzed. The analyte should be added to the collection matrices in the field at the time of the study. After fortification and evaporation of the delivery solvent, the fortified matrices should be exposed to ambient conditions and attached to air pumps. The pumps should be operated in clean air at a flow rate, and for the length of time, equivalent to the field samples.

Patches. An appropriate number of controls, low-level fortifications, and high-level fortifications should be prepared and analyzed. The number of patches is irrespective of whether the worker wears 1 patch or 22 patches. However, if some patches are covered by clothing (inside patches versus outside patches), a separate set of fortified patches may be prepared and covered by clothing during the exposure period at the control site. If necessary, fortified outside patches can be substituted for fortified inside patches as they represent the worst case. However, this approach is not recommended as it can result in a considerable overestimation of losses during the sample collection period which, in turn, results in an overestimate of dermal exposure (outside patch field recovery used for inside patch correction).

Whole Body Dosimeters (WBDs). Preferably, investigators should collect field samples from test subjects in a manner that reflects the exposure to various regions of the body (e.g., field samples should be sectioned into samples of the following: arms, legs, front torso, back torso). A field quality control regimen should reflect this type of study protocol by using pieces of test garments for fortification as field recovery samples. Investigators must use discretion when preparing samples. For example, investigators could split a

garment designated for QC purposes into samples that are reflective of the field samples (e.g., arms or legs) or into smaller fabric swatches (e.g., 100 cm^2) and fortify the individual samples. However, several issues must be considered when attempting to prepare such samples.

First, fortification levels must reflect the relative size of the QC fabric swatch samples as opposed to a typical sample (i.e., the fortification level should be similar to the anticipated field sample levels on a per area basis as analytical background may be important in the data interpretation). Second, the total solvent extraction volume should be proportional to the surface area for both the field exposure samples and the field recovery samples (e.g., if a solvent volume of 1,000 mL is used to extract a 2,000 cm^2 section of a field exposure sample, then a solvent volume of 50 mL should be used to extract a 100 cm^2 field recovery swatch). Third, QC sample swatches must be generated from the same production lots of dosimeters and prepared in the same manner (e.g., pre-extraction) as the monitors used to collect the actual field samples. Finally, the quantitation limit for each particular matrix must be considered when specifying fortification levels (e.g., the determination of low fortification levels when prorating based on the surface area of the sample). The use of fabric swatches will conserve considerable storage space and solvent usage. The similarity of recoveries should be established during method development and validation. If it is established that the fortifying of fabric swatches yields results similar to whole sections of the WBD, it is acceptable to use patches for field recovery samples.

Gloves, Socks, Undergarments, Head Bands, etc. Exposed clothing items, such as gloves (if used as a dosimeter), should be fortified at both the low level and the high level. Covered items, such as undergarments, may only need to be fortified at the low level, covered, and exposed to the elements at the control site.

Hand Rinses; Urine/Blood. It is not appropriate to expose hand rinse or biological samples to the environment during the field phase of a study because they are collected, processed, and stored immediately, without significant exposure to the elements. However, all samples should be handled using the same procedures as the actual field samples. For example, fortified hand rinse solutions should be "set out," for as long as it takes to conduct a hand wash (10-15 minutes), prior to storage or packing for shipment. If urine or blood is collected for biological monitoring, it is recommended that three samples of control (nonparticipant) urine or blood be fortified with two levels of the urinary analyte (parent or metabolite(s), whichever is appropriate) for each experimental site. These fortifications may be made just prior to going to the field, carried into the field during the course of a work cycle, exposed in a manner similar to participants' urine or blood, and stored and shipped with experimental samples. If stability of the analyte(s) has been rigorously established prior to study initiation, investigators may choose to reduce or eliminate this component from the study design because it is a burdensome task.

3.1.2   Field Recovery for Environmental Residues

This section describes how to generate field recovery data for any method designed to quantify transferable residues. Like the situation described above for hand rinses, dislodgeable foliar residue samples are collected, processed, and stored immediately without significant exposure to environmental elements. The objective of dislodgeable residue field recovery samples is to account for losses that occur from the time of collection through transportation, storage, and analysis. This can be accomplished by fortifying blank extract solutions at various sampling intervals and then transporting, storing (both in the field and in the laboratory), and analyzing the field recovery samples in conjunction with the foliar and soil residue samples. The blank extract solutions are prepared in the same manner as the dislodgeable foliar residue samples except that residue free foliage is used. On the other hand, residue free, sampling media, such as the PUF from the PUF roller, cloth from the Dow Drag Sled, or soil should be fortified directly. The recommended sampling intervals for dissipation studies are discussed in Part B, Chapter 2 - Study Design.

The frequency of preparing field recovery samples to correct for potential dislodgeable residue sample losses will be dictated by the stability of the pesticide in the extract solution as determined during the pre-field storage stability study. Pesticides that are relatively stable in the extract solution under the anticipated storage conditions will require that field recovery samples be prepared at less frequent sampling intervals than pesticides that are less stable. All foliar or soil residue samples collected in conjunction with or subsequent to each set of field recovery samples should be transported, stored, and analyzed concurrently with the field recovery samples. An alternative to analyzing the dislodgeable residue samples concurrently with the field recovery samples is to analyze them prior to the field recovery samples but in conjunction with concurrent laboratory recovery samples. A complete set of field recoveries should consist of at least one blank control sample and three or more each of a low-level fortification and a high-level fortification. The low- and high-level fortifications should be in the range of the anticipated levels of the residues in the field.

3.1.3   Travel Recoveries

Travel recoveries refer to experiments conducted to determine the stability of the analyte on each sampling matrix during shipment and possibly storage. Travel recoveries are optional and are left up to the discretion of the investigator. These recovery samples are prepared concurrently with the field portion of the study. They are then shipped and stored with the appropriate experimental samples. However, appropriate steps should be taken to ensure that commingling of these samples does not occur (i.e., travel recovery and field samples should be kept in separate containers within the shipping or storage unit to prevent cross contamination). The travel recovery samples are different than the field recovery samples in that they are not exposed to the environmental conditions during the sample collection period. Thus, the results of the travel recovery samples reflect losses that may occur during shipment and storage only as opposed to those that

occur during sample collection, shipment and storage. It is suggested that one set of travel recovery samples be prepared for each experimental site to aid in the interpretation of losses that may occur in field recovery samples. If field recovery samples indicate no significant losses, the travel recoveries do not need to be analyzed.

3.1.4   Fortification Solutions

The importance of valid field recovery data (and thus, the ability to accurately fortify field recovery samples with a known mass of active ingredient) to the overall success of these studies cannot be overemphasized. It is recommended that a solution of the neat analyte, in the same form as applied, usually in an organic solvent, be used for fortifying all substrates. Such a solution is also recommended for fortifying hand rinse solutions and control urine samples, depending on the analyte. It is unlikely that the organic solvent used to prepare the fortifying solution will change extraction characteristics of the analyte because an extremely low volume of a concentrated solution is typically used to fortify samples. The concentration may have to be adjusted for fortifying at multiple levels. A description of the fortification procedures must be added to the final report.

3.1.5   Control Site

Blank samples and field recovery samples are to be prepared at a "control site" that is upwind and a reasonable distance from the treatment site to avoid potential contamination of the samples during application and/or postapplication activities. Good planning and adequate resources are required to have the control site established, and all fortified and control collection devices "running" while the field monitoring portion of the study is in progress. The exposure time at the control site should approximate the field sampling time as closely as possible.

3.2   Field Data Collection and Documentation

Comprehensive and accurately written and photographic field records are critical to evaluating the representativeness of a study's design and interpreting study results. As such, it is recommended that in addition to maintaining written documents as required by GLP, researchers photograph and/or videotape the various activities associated with sample collection (e.g., sample collection, sample storage, etc.).

The data collection requirements for the following phases of a field trial are described below.

- Study Site Characteristics;
- Application Equipment and Procedures;

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- Climatological Data;
- Sampling Equipment and Techniques; and
- Exposure Activity Patterns.

3.2.1   Study Site Characteristics

A reasonable/general description (e.g., a diagram and/or text) of each study site (indoor and outdoor) must be included as part of the protocol and study report submitted to the Agency. Any equipment that is permanently situated onsite that might influence the exposure measurements must be described in the field notes, including such items as:

- Blocking and shade cloth arrangements in greenhouses;
- Ventilation systems; and
- Automated control systems (e.g., greenhouse climate, irrigation, etc.).

Soil must be characterized where applicable. Soil characterizations must include texture and classification.

3.2.2   Application Equipment and Procedures

Application procedures must be thoroughly documented. Sprayer calibration and formulated pesticide product samples as well as photographic records are essential in determining whether or not an application is valid. Valid application procedures are essential to any acceptable study.

All application equipment used in a study must be calibrated. Application rate verification (e.g., deposition coupon, calibration, inventory) is generally recommended and should be discussed with the Agency during the development of the study protocol.

Any equipment that is used during a study such as mechanized harvesting equipment, tractors, hand tools, and stationary packing equipment must be completely documented. This description should include the following characteristics:

- Equipment capacity (mechanical harvesters, cherry pickers, etc.);
- Attainable height (e.g., ladder height, cherry picker height, etc.);
- Equipment model and operating parameters;
- Hand tools (type, size, uses); and
- Picking site logistics and ergonomic factors.

3.2.3   Climatological Data

The following types of data are required, where appropriate, for dislodgeable foliar residue and soil residue dissipation studies (also, indoor studies, as appropriate):

- •   Air temperature (indoor also);
- •   Precipitation (natural and irrigation);
- •   Relative humidity (indoor also); and
- •   Air exchange should be described for indoor studies.

Study conditions and the physical/chemical properties of the pesticide(s) may require additional information be collected.

The Agency will note these data on a case-by-case basis.

The use of climate data from offsite sources is acceptable if large variances do not exist. Investigators must use their discretion when identifying sources of alternative data. For example, climatological data from weather station observatories near a study site can serve as an acceptable alternative for weather information monitored onsite if it can be determined that large variances do not exist between the climatic conditions at the two locations (e.g., spot check similarities at various intervals). Onsite monitoring of rainfall is recommended. If alternative data are used, a general discussion regarding why they were used must be included in the study report submission.

A relevant report of the effect of temperature and humidity on residue decay (and data reporting for that matter) may be found in Popendorf, W.J. and Leffingwell, J.T.: Natural Variations in the Decay and Oxidation of Parathion Foliar Residues. J. Ag. and Food Chem. 26(2):437-441, 1978.

3.2.4   Sampling Equipment and Techniques

A complete description of sampling techniques utilized by an investigator throughout the course of any study must be provided. The following types of information are required:

- •   Sampler make and model information; and
- •   Dosimeter design and attachment mechanisms.

Any sample collection equipment used in a study by an investigator must be validated and/or calibrated. Examples include: personal sampling pumps must be calibrated using a device that is traceable to or is a primary standard (e.g., bubble meter or "Buck-Type" calibrator); thermometers used in any study must be traceable to a National Institute of Standards and Technology (NIST) primary standard; and weights used to calibrate analytical balances must also be traceable to NIST primary standards. The validation/calibration should be described in standard operating procedures (SOPs).

A complete description of any sampling regimens utilized by an investigator throughout the course of any study must be completely described. The following types of information are required:

- Dosimeter location on each test subject;
- DFR Sampling regimen (e.g., Iwata Method for Trees); and
- Stationary Air Sampler Placement.

4.0 LABORATORY ANALYSIS OF COLLECTED SAMPLES

By the time the collected samples arrive at the laboratory, the study investigator should have a reliable analytical method in place. The basis for any modern analytical method is the specificity (e.g., separation). Instrumental methods that are both sensitive and stable should be developed. The analyte(s) of interest should be readily separated from the unidentified analytical response (UAR) by units of time, wavelength, etc., that are large enough to allow for accurate resolution and quantification of the analyte of interest. The lowest level analytical (i.e., calibration) standard should produce a signal that is at least three times greater in magnitude than the baseline background noise. In other words, the instrumental signal to noise ratio must be ≥ 3 for the lowest standard (Taylor, 1987).

4.1 <u>Instrument Performance</u>

Instrument performance must be monitored regularly to ensure the reliability of the measurements. Several techniques that can be used to establish instrument performance include, but are not limited to: (1) daily comparison of peak areas of analytical standards via linear regression, (2) calculation of a correlation coefficient for a particular standard curve, or (3) judicious selection of internal standards. Investigators must establish their own guidelines for determining whether an instrument is functioning properly, as this determination is dependent upon the analytical method, instrument operating parameters, and background levels observed and/or anticipated in the samples. Investigators should describe in detail any procedures used to monitor instrument performance on a routine basis.

Determining the proper instrument operational quality control procedures is difficult. Investigators must develop operational standards that are pertinent to the pesticide(s) being studied (e.g., detector response patterns affect calibration techniques). Investigators must also develop criteria for scrutinizing daily method performance data.

4.2   Calibration Techniques

Investigators should describe all techniques used to calibrate instruments and generate calibration curves. Data integration, besides enabling investigators to manipulate and interpret data in a variety of ways (i.e., various peak integration techniques), also typically generate calibration curves as well as calculate and summarize results. Several options for instrument calibration are usually available in each system (e.g., linear regression for all points, point to point calibration, average values based on multiple analysis, etc.). Investigators must be careful to consider the response patterns of a particular instrumental system (i.e., linear, exponential, threshold, etc.) prior to selecting a means to generate the calibration curve. Technique(s) employed by investigators to calibrate an instrument should be described in any submission to the Agency.

4.3   Concurrent Laboratory Recovery

Laboratory recovery samples are analyzed in the analytical laboratory concurrently with the field samples to confirm or determine the recovery efficiency of the analyte from substrates. Laboratory recovery samples are typically fortified with analytical standards or formulation and reflect losses that occur during laboratory operations (extraction, cleanup, analytical measurement, etc.). They are not intended to account for losses that occur during sample collection, shipping or storage. It is recommended that a minimum of ten percent of the field samples be represented by a laboratory recovery sample for each analytical batch or run. These laboratory recovery samples should cover the range of concentrations anticipated in field samples.

Laboratory recovery samples can be either fortified in the laboratory or in the field (i.e., field recovery samples). It is recommended, however, that the field fortified samples be used as concurrent laboratory samples, whenever possible. When used in this manner, the field fortified samples can be used to correct the field samples for both losses in the field and laboratory. However, if the investigator is not confident of the environmental fate of the compound in the field and during storage, samples generated in the laboratory should be used to identify where the losses may have occurred (i.e, field or analytical method).

5.0   GOOD LABORATORY PRACTICES

Quality assurance requirements may be found in the Good Laboratory Practice (GLP) Standards (40 CFR 160.35). These regulations "define the function of the quality assurance unit (QAU) in regulated studies

as that of ensuring managers that all aspects of the facility, personnel, performance, recordkeeping, and reporting are consistent and in compliance with the regulations. The objective of the regulations is to ensure users...of (the generated) information...of accuracy and to ensure integrity of study conduct and reported results according to specifications in the GLPs."

Sample storage and shipment procedures as well as the chain-of-custody system must be documented in full. Any GLP exceptions need to be accepted by EPA in the protocol (e.g., container storage).

5.1   Sample Storage and Shipment

The study investigator should be responsible for demonstrating the stability of the samples under the storage duration and conditions used.

5.2   Chain-of-Custody

"Documentation of chain-of-custody is necessary to provide information concerning the handling of test substances, reference substances, control samples, and treated samples within the analytical laboratory. Chain-of-custody includes not only the receipt of a substance, but also from whom that substance was received and the condition of the sample upon receipt. Once a substance or sample is in the possession of the analytical laboratory, the storage conditions must be documented.
Chain-of-custody documentation provides a 'paper trail' that tracks the removal of these items from storage for any reason: weighing, mixing, spraying, sampling, processing, assay, or shipment" (Garner et al. 1992). Chain-of-custody is required under 40 CFR 160.81.

REFERENCES FOR PART C

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